

REMARKS

Entry of the foregoing, reexamination and reconsideration of the subject application, as amended, pursuant to and consistent with 37 C.F.R. § 1.116, are respectfully requested in light of the remarks which follow.

I. Claim Amendments

By the foregoing amendments to the claims, claim 1 has been amended and claims 3, 7, and 8 have been canceled.

In particular, claim 1 has been amended to recite particular pentoses, pentose esters, and acid phosphatases. The pentoses and pentose esters are described at least at original claim 3. Support for acid phosphatase derived from *Shigella flexneri* can be found at least at original claim 8; support for acid phosphatase derived from *Escherichia blattae* can be found at least at page 7, lines 9-10 of the specification.

The amendments to the claims, including cancellation of claims, have been made without prejudice or disclaimer to any subject matter recited or canceled herein. Applicants reserve the right to file one or more continuation and/or divisional applications directed to any canceled subject matter. No new matter has been added, and entry of the foregoing amendments to the above-identified application are respectfully requested.

II. Response to Claim Rejections Under 35 U.S.C. § 103

Claims 1-8 have been rejected under 35 U.S.C. § 103(a) as purportedly being unpatentable over Tanaka et al. (Org. Biomol. Chem. 1:2833-2839 (July 9, 2003)) in view of Gross et al. (J. Am. Chem. Soc. 105:7428-7435 (1983)). This rejection is respectfully traversed.

To expedite prosecution in the present application, and not to acquiesce to the Examiner's rejection, the claims have been amended as described above. Applicants submit that the cited references, taken alone or together, do not teach or suggest the subject matter of the present claims, for at least the following reasons.

The present inventors have unexpectedly discovered a process comprising phosphorylation of a free pentose with the use of an acid phosphatase. In the present application, the inventors studied the substrate specificity of acid phosphatase for various pentoses, and surprisingly discovered that pentoses capable of serving as substrates for acid

phosphatase have a particular steric structure on the 5-OH of the reacting site and comprise 4- and 3- OH. As recited in the claims, the present inventors found that suitable pentoses are classified in (3S, 4R) or (3R, 4S). More particularly, suitable pentoses for phosphorylation using acid phosphatase derived from *Shigella flexneri* or *Escherichia blattae* are ribose, arabinose or 2-deoxyribose.

In contrast to the present application, Tanaka et al. describe selective phosphorylation of glucose and do not teach or suggest phosphorylation of a free pentose. In this regard, Applicants note that whether a compound is suitable as an enzyme substrate depends on the substrate specificity of the enzyme, which is highly unpredictable. Even where two compounds may have some apparent structural similarity, those two compounds are not necessarily effective substrates for a common enzyme. Thus, even if glucose was known as a substrate for an acid phosphatase, the reactivity of pentose could not be predicted, because the substrate specificity of the acid phosphatase had not been tested and was not known in the art. In particular, the specificity of pentose phosphate derived from *Shigella flexneri* or *Escherichia blattae* for ribose, arabinose and 2-deoxyribose was not known in the art prior to the present invention.

As noted above, the present inventors found that suitable pentoses are classified in (3S, 4R) or (3R, 4S). On the other hand, pentoses not suitable as substrates for acid phosphatase are classified in (3R, 4R). In contrast, hexoses did not follow this pattern. Further, as shown in Example 2 (FIG. 1) of the present application, deoxyribose is poorly phosphorylated under conditions similar to the glucose phosphorylation conditions of Tanaka et al. (i.e. 100mM glucose, 100 mM sodium pyrophosphate), demonstrating that a one-carbon difference in carbon number can drastically change the reactive yield (glucose: not less than 30%, deoxyribose: 1%). Thus, although hexose and pentose both have hydroxyl groups and high polarity, a person of ordinary skill in the art would not have reasonably predicted that pentose, particularly ribose, arabinose and 2-deoxyribose, would be an effective substrate for acid phosphatase derived from *Shigella flexneri* or *Escherichia blattae*.

Tanaka et al. also describes selective phosphorylation of inosine. However, as noted in the prior response, Ishikawa et al., Protein Eng. 15:539-543 (2002) shows the presence of hypoxanthine coordinated first place of inosine is important for the recognition of inosine as a substrate, for orientation control and for the reaction of inosine with acid phosphatase (see FIG.1, 2nd paragraph of page 541, 2nd paragraph of page 542, and Table 2 of Ishikawa et al.).

Accordingly, a person of ordinary skill in the art would not have expected free pentose to be useful as an acid phosphatase substrate, because free pentose is not modified by hypoxanthine.

In addition, Ishikawa et al. disclose that "the inosine was placed on a depression surrounded by Leu16, Ser71, Ser72 and Glu104"; "the position of the base is affected by the conformation and the position of the ribose, it seems most plausible to place it near Ser71 and Glu104"; and one of the possible inosine-binding modes is shown in Fig.1(2nd paragraph of page 541). Ishikawa et al. further disclose that "To maximize the possibility of the formation of an aromatic-aromatic interaction, the introduction of the largest amino acid, tryptophan, to the above four positions seemed promising, and thus the following four mutations were designed: Leu16→Trp, Ser71→Trp, Ser72→Trp and Glu104→Trp. The K_m values and the relative activities of crude extracts of the resultant mutants are shown in Table II" (2nd paragraph of page 542). It was expected that replacements of Leu16, Ser71, Ser72 and Glu104 with Trp generate the interaction between the aromatic ring of Trp and a base of inosine. As a result, the K_m values of an enzyme modified at Leu16 and Ser72 were improved compared to an unmodified enzyme. The results described above show that the positions of the inosine bases were good for the reaction of enzyme.

On the other hand the K_m values of an enzyme modified at Ser71 and Glu104 were not so improved compared to that of Leu16 and Ser72 respectively. Therefore, the result of the improvement of K_m value of an enzyme modified at Leu16 and Ser72 shows that the inosine was placed on a plausible position. On the other hand Ser71 and Glu104 near the plausible place, Leu16 and Ser72, properly act as a side-chain to recognize the base, indirectly. Thus, Leu16, Ser71, Ser72 and Glu104 are expected to interact with bases of nucleoside. And K_m values of modified enzymes produced by replacing Leu16, Ser71, Ser72 and Glu104 were improved.

In addition, it is clear that the authors of the cited Ishikawa et al. Protein Engineering reference recognize the influence of inosine, as shown in Ishikawa et al., US 6,987,008 B1. This patent discloses that "since it is expected that Ser72 most strongly interacts with a base of nucleoside in the binding mode model"(page 7 line 39 to 41), "It is also expected that replacements of Leu16, Ser71, Ser73 and Glu104 with Phe, Tyr and Trp generate the π - π interaction between the aromatic rings of the replaced amino acid residues and a base of nucleoside"(page 7, line 50 to line 52).

Furthermore, the Ishikawa et al. Protein Engineering reference provides lists demonstrating reduced Km value, as follows:

	Km
L16W/G74D/I153T	33
S71W/G74D/I153T	75
S72W/G74D/I153T	30
E104W/G74D/I153T	67
S72F/G74D/I153T	20
S72Y/G74D/I153T	30
S72D/G74D/I153T	38
S72E/G74D/I153T	40
S72V/G74D/I153T	41
S72M/G74D/I153T	46
S72T/G74D/I153T	50
G74D/I153T	77

Therefore, the following mutations were designed:

By Leu16→Thp, Gly74→Asp and Ile153→Thr, the Km value is 33,
by Ser71→Thp, Gly74→Asp and Ile153→Thr, the Km value is 75,
by Ser72→Thp, Gly74→Asp and Ile153→Thr, the Km value is 30,
by Glu104→Thp, Gly74→Asp and Ile153→Thr, the Km value is 67,
by Ser72→Phe, Gly74→Asp and Ile153→Thr, the Km value is 20,
by Ser72→Tyr, Gly74→Asp and Ile153→Thr, the Km value is 30,
by Ser72→Asp, Gly74→Asp and Ile153→Thr, the Km value is 38,
by Ser72→Glu, Gly74→Asp and Ile153→Thr, the Km value is 40,
by Ser72→Val, Gly74→Asp and Ile153→Thr, the Km value is 41,
by Ser72→Met, Gly74→Asp and Ile153→Thr, the Km value is 46,
by Ser72→Thr, Gly74→Asp and Ile153→Thr, the Km value is 50, and
by Gly74→Asp, Ile153→Thr, the Km value is 77.

By the same token above, Leu16, Ser71, Ser72 and Glu104 are expected to interact with bases of nucleoside. And Km values of modified enzymes produced by replacing Leu16, Ser71, Ser72 and Glu104 are improved.

In summary, Ishikawa et al. demonstrate that the presence of hypoxanthine is important in orientation control for the activity of inosine.

In addition, Applicants respectfully disagree with the Examiner's position that "the enzyme studied by Ishikawa is that from *Escherichia blattae*, which is not the same enzyme as that taught by Tanaka et al." Applicants acknowledge that the enzyme taught by Ishikawa et al. is from *Escherichia blattae*, and the enzyme taught by Tanaka et al. is from *Shigella flexneri*. However, the reacting configuration of the enzymes is identical. It is known in the art that sequences having a high degree of sequence identity, even if derived from different organisms, are highly likely to share a common function. Furthermore, the enzymes will generally have a common active site. Applicants' argument is supported by the following figures and tables, which have been submitted herewith.

Fig.1-1 shows alignment of the amino acid sequence of the aspartate aminotransferase derived from pig(1AJS) with the amino acid sequence of the aspartate aminotransferase derived from chicken (2CST) by GENETYX-WIN.

The homology of the alignment of 1AJS and the alignment of 2CST is 82%.

Fig.A shows a structure of the active site of 1AJS.

Fig.B shows a structure of the active site of 2CST.

Data A shows the search screen for 1AJS by "Protein Data Bank (<http://www.rcsb.org/pdb/home/home.do>)" displayed on the computer screen.

Data B shows the search screen for 2CST by "Protein Data Bank (<http://www.rcsb.org/pdb/home/home.do>)" displayed on the computer screen.

Tyr226 of 1AJS corresponds to Tyr226 of 2CST, Phe360 of 1AJS corresponds to Phe360 of 2CST, Agr386 of 1AJS corresponds to Agr386 of 2CST, Asn194 of 1AJS corresponds to Asn194 of 2CST, Trp140 of 1AJS corresponds to Trp140 of 2CST, Agr292 of 1AJS corresponds to Agr292 of 2CST, Tyr70 of 1AJS corresponds to Tyr70 of 2CST, Lys258 of 1AJS corresponds to Lys258 of 2CST, and PLP of 1AJS corresponds to PLP of 2CST. Therefore when the active site of 2CST is common with the active site of 1AJS, both the function and the structure of the active site is the same.

Kiyoshi Kondo et al. (Vol.262, No.18, pp.8848-8659, June 1987; submitted herewith) shows that "The complete amino acid sequences of the cytosolic aspartate aminotransferases from pig (6,7) and chicken (8)," "comparative studies indicate that the degree of interspecies sequence identity of the homotopic isoenzymes is more than 80%," and "X-ray studies on the pig cytosolic(14), chicken cytosolic(15,16), and chicken mitochondrial(17) enzymes revealed that their three-dimensional structure are virtually the same." In summary, when enzymes share a high degree of sequence identity and the same function, their three-dimensional structure is virtually the same. When this principle is applied to the present case, the following can be shown.

Fig.1-2 shows an alignment of the amino acid sequence of the *Escherichia blattae* and the *Shigella flexneri* acid phosphatase enzymes. As shown in **Fig.1-2**, the degree of sequence identity between the enzymes is relatively high, 84.4%.

Fig.C shows a three-dimensional structure of the active site of *Escherichia blattae*.

Accordingly, a person of ordinary skill in the art would reasonably predict that the active site of the *Shigella flexneri* acid phosphatase would have the same three-dimensional structure as that of the *Escherichia blattae* acid phosphatase. Therefore, the function of *Escherichia blattae* acid phosphatase is also common with that of *Shigella flexneri* acid phosphatase. Thus, based on Ishikawa et al., a person of ordinary skill in the art would not have expected free pentose to be useful as a substrate for either *Escherichia blattae* acid phosphatase or *Shigella flexneri* acid phosphatase.

With regard to the unpredictability of phosphorylation reactions with free pentose as a substrate, Applicants submit that solutions of reducing sugars are known to take various structures with furanose, pyranose and aldehyde. In order for a phosphorylation reaction to proceed, a sugar must be in the form of a structure with primary alcohol. Applicants' position is supported by, for example, WO98/39347 (submitted herewith). Sugar is phosphorylated at a primary alcohol thereof. For example, a position of primary alcohol in glucose is 6 and in ribose is 5. Therefore glucose will be phosphorylated at position 6, and ribose will be phosphorylated at position 5. For a position of primary alcohol moiety in aldopentoses including ribose is 5, aldopentoses have to consist of furanose. If an aldopentose consists of pyranose, it cannot be phosphorylated because of the absence of a primary alcohol at position 5.

Tanaka et al. teaches a phosphorylation reaction on inosine as well as glucose. Inosine consists of only furanose because of the presence of a hypoxanthine moiety. On the other hand, pentoses are known to have equilibrium mixtures of furanose and pyranose. As described above, where a pentose consists of pyranose, such a pentose could not be phosphorylated. However, where a pentose consists of furanose, such a pentose could be phosphorylated. Additionally, Ishikawa et al. teaches that the presence of hypoxanthine is important, as described above. Therefore, since a free pentose has no large moiety such as the hypoxanthine of inosine, the phosphorylation of a free pentose should not simply be considered as analogous to the phosphorylation of inosine. Furthermore, glucose consists of pyranose(100%). That is glucopyranose can be phosphorylated since it has a primary alcohol at position 6. Therefore, even if Tanaka et al. teaches the phosphorylation of glucose, the skilled artisan would not expect the phosphorylation of a free pentose in the present invention.

The Declaration Under 37 C.F.R. § 1.132 of Keiichirou Kai, submitted herewith, clearly shows that while some pentoses and hexoses are phosphorylated using acid phosphatase, some are not.

Table.3-1 and **Fig.3-1**, respectively, show the summary of the results of effectivity of free pentoses, 2- deoxy- D-ribose, 2- deoxy- L-ribose, D-ribose, D-xylose, D-arabinose, L-arabinose and D-lyxose (see the figures and tables enclosed herewith). Example A in the Declaration shows that 2-deoxyribose, D-ribose and arabinose can be phosphorylated, on the other hand D-xylose and D-lyxose can not be phosphorylated from various pentoses. These results demonstrate that even if these pentoses all have the ability to take free configurations in view of absence of a large moiety, their reactivity is remarkably different from one another.

Table.3-2 and **Fig.3-2** respectively show the summery of the results of effectivity of free hexoses, D-glucose, 2- deoxy- D- glucose, D-mannose and D-galactose, D-sorbitol, D-myo-inositol, D-glucosamine and D-glucono-1,5-lactone (see the tables and figures enclosed herewith). Example B in the Declaration shows that D-glucose, D-mannose, D-sorbitol and D-glucosamine can be phosphorylated, on the other hand D-galactose, D-myo-inositol and D-glucono-1,5-lactone cannot be phosphorylated from various hexoses.

It is true that Tanaka et al. teaches the reaction of glucose as well as inosine. However, as shown in Declaration Example B, the reactivities of hexoses vary with the type thereof. Additionally, among sugar alcohols from hexose, D-sorbitol can be phosphorylated

but D-myo-inositol cannot. Also among hexose derivatives, D-glucosamine can be phosphorylated but D-glucono-1,5-lactone cannot. Therefore, some hexoses can be phosphorylated, and some hexoses cannot be phosphorylated, even if one hexose is a companion of another. Further, although the pentose differs from the hexose in number of carbons, it is still difficult to forecast which pentose will react based on the behavior of other pentoses. Thus, even if Tanaka et al. teaches that the reaction proceeds on glucose, the skilled artisan would not expect the reaction proceeds of a free pentose of the present invention.

Regarding the reacting site of pentose, Tanaka et al. disclose that "PhoN-Sf catalyses the phosphorylation of inosine to inosine-5'-monophosphate(5'IMP), whereas PhoN-Se synthesizes both 5'IMP and inosine-3'-monophosphate(3'IMP)"(page 2834 second paragraph line10 to line 13). The position of the hydroxyl group in inosine phosphated was different, corresponding to the difference between PhoN-Sf and PhoN-Se. Therefore, it is obvious that the position of phosphorylated hydroxyl group in pentose is different depending on the kind of the genus of the acid phosphatase to be employed. This is supported at least by Y. Asano et al. Journal of Molecular Catalysis B: Enzymatic 6(1999)pp.274 Table 1. Accordingly, a person of ordinary skill in the art would have reasonably predicted that not all pentoses could be selectively phosphorylated at the position of five. In particular, the skilled artisan would not have reasonably expected that *Shigella flexneri* or *Escherichia blattae* enzyme would be suitable for selectively phosphorylating ribose, arabinose or 2-deoxyribose at position 5.

Finally, as noted in the prior response, Gross et al. does not remedy the serious deficiencies of Tanaka et al. In particular, Gross et al. does not teach or suggest preparing a pentose-5-phosphate ester. The process described in Gross et al. and cited by the Examiner (right column of page 7429, the second line from the bottom) is a process for producing ribose-5-phosphate ester by using ATP as phosphate donor and ribokinase as enzyme. Thus, the process disclosed in Gross et al. is not related to the present invention.

For at least these reasons, the present process would not have been obvious to a person of ordinary skill in the art, and Applicants thus respectfully request reconsideration and withdrawal of the 35 U.S.C. § 103(a) rejection.

CONCLUSION

From the foregoing, favorable action in the form of a Notice of Allowance is respectfully requested and such action is earnestly solicited.

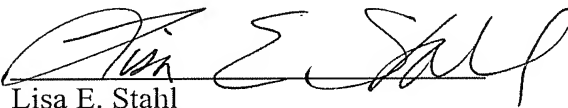
In the event that there are any questions related to this response, or the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney at the below-listed telephone number concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

BUCHANAN INGERSOLL PC

Date: December 8, 2008

By:

A handwritten signature in black ink, appearing to read 'Lisa E. Stahl', written over a horizontal line.

Lisa E. Stahl

Registration No. 56,704

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DOI 10.2210/pdb1ajs/pdb

REFINEMENT AND COMPARISON OF THE CRYSTAL STRUCTURES OF PIG CYTOSOLIC ASPARTATE AMINOTRANSFERASE AND ITS COMPLEX WITH 2-METHYLASPARTATE

Rhee, S., Silva, M.M., Hyde, C.C., Rogers, P.H., Metzler, C.M., Metzler, D.E., Arnone, A.

Rhee, S., Silva, M.M., Hyde, C.C., Rogers, P.H., Metzler, C.M., Metzler, D.E., Arnone, A. (1997) Refinement and comparisons of the crystal structures of pig cytosolic aspartate aminotransferase and its complex with 2-methylaspartate. *J.Biol.Chem.* 272: 17293-17302

[Abstract] Pubmed

History Deposition 1997-05-08 Release 1997-08-20

Experimental Method Type X-RAY DIFFRACTION Data [EDS]

Resolution[Å] R-Value R-Free Space Group
1.60 0.173 (obs.) n/a P 2₁ 2₁ 2₁

Length [Å] a b c
Angles [°] alpha beta gamma
130.80 90.00 55.80
90.00 90.00

Molecular Description Asymmetric Unit

Polymer: 1 Molecule: ASPARTATE AMINOTRANSFERASE Chains: A EC no. 2.6.1.1
Other Details: THE COENZYME PYRIDOXAL 5'-PHOSPHATE IN SUBUNIT A FORMS A SCHIFF BASE WITH THE SUBSTRATE ANALOG 2-METHYLASPARTATE, AND FORMS THE EXTERNAL ALDIMINE. BUT DUE TO CRYSTAL LATTICE PACKINGS THE COENZYME IN SUBUNIT B IS STILL IN THE INTERNAL ALDIMINE WITH THE SIDE CHAIN OF LYS 258.
Polymer: 2 Molecule: ASPARTATE AMINOTRANSFERASE Chains: B EC no. 2.6.1.1

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DOI 10.2210/pdb2cst/pdb

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Title
CRYSTAL STRUCTURE OF THE CLOSED FORM OF CHICKEN CYTOSOLIC ASPARTATE AMINOTRANSFERASE AT 1.9 ANGSTROMS RESOLUTION

Authors Malashkevich, V.N., Strokopytov, B.V., Borisov, V.V.

n/a (1995) Crystal structure of the closed form of chicken cytosolic aspartate aminotransferase at 1.9 Å resolution. *J.Mol.Biol.* 247: 111-124Primary Citation
[Abstract] Pubmed

History Deposition 1994-09-06 Release 1994-11-30

Experimental Method Type X-RAY DIFFRACTION Data N/A

Resolution[Å]	R-Value	R-Free	Space Group
n/a	n/a	n/a	P 2 ₁ 2 ₁ 2 ₁

Length [Å]	a	56.40	b	126.00	c	124.30
Angles [°]	alpha	90.00	beta	90.00	gamma	90.00

Molecular Description
Asymmetric Unit

Polymer: 1 Molecule: ASPARTATE AMINOTRANSFERASE Chains: A,B EC no.: 2.6.1.1

Structure Weight: 92443.23

Classification Transferrase (aminotransferase)

Images and Visualization

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Fig .
A

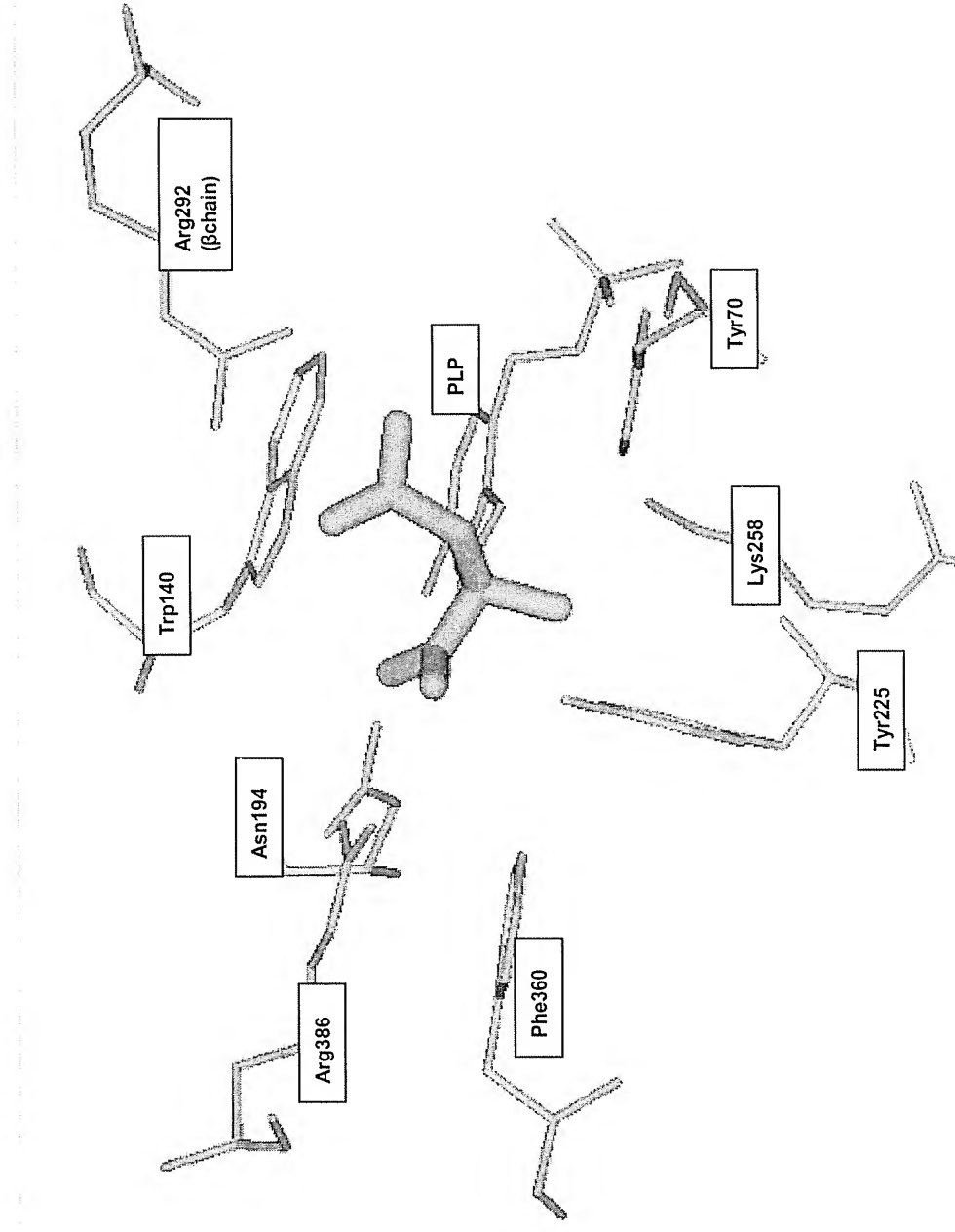
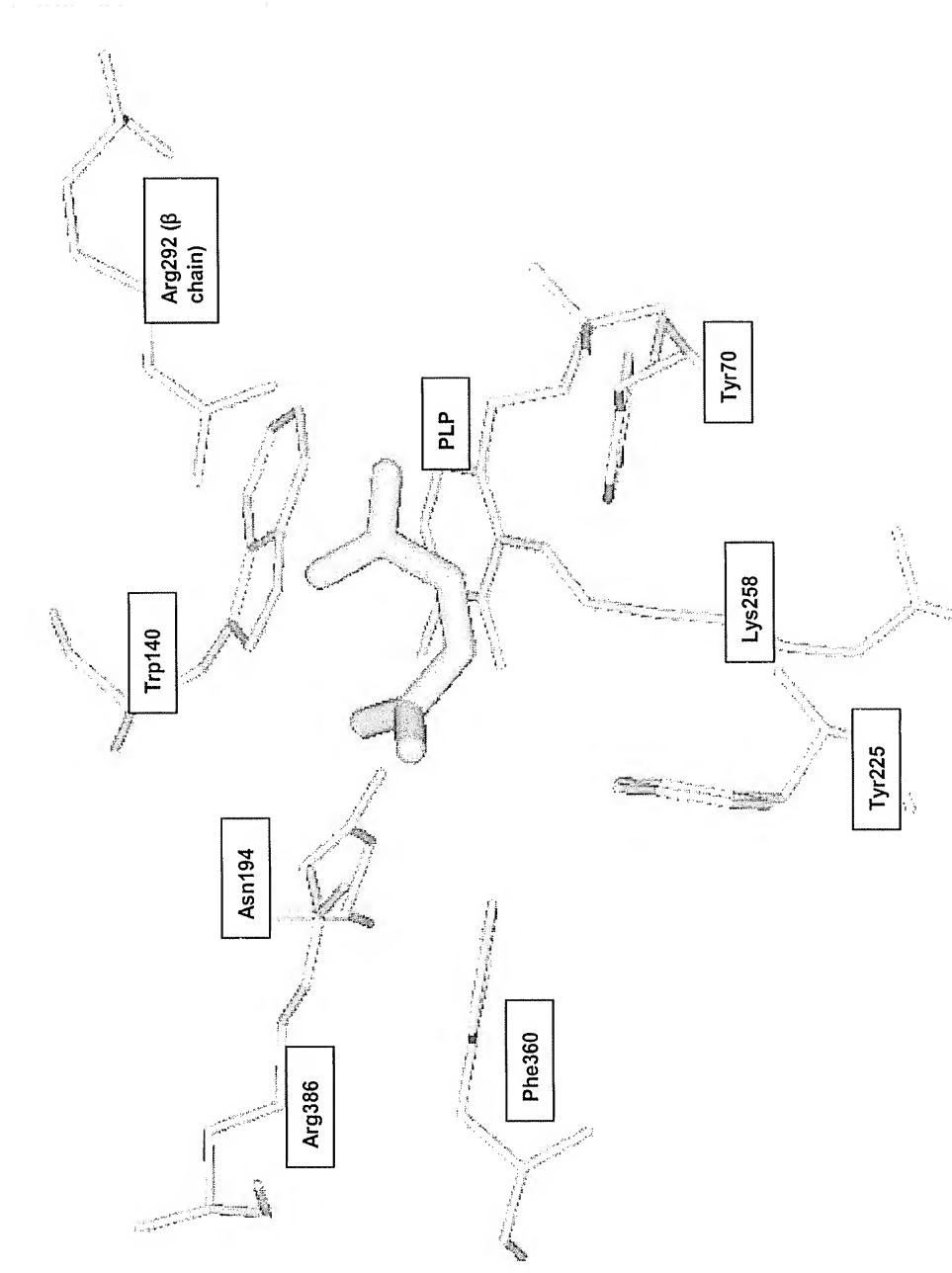
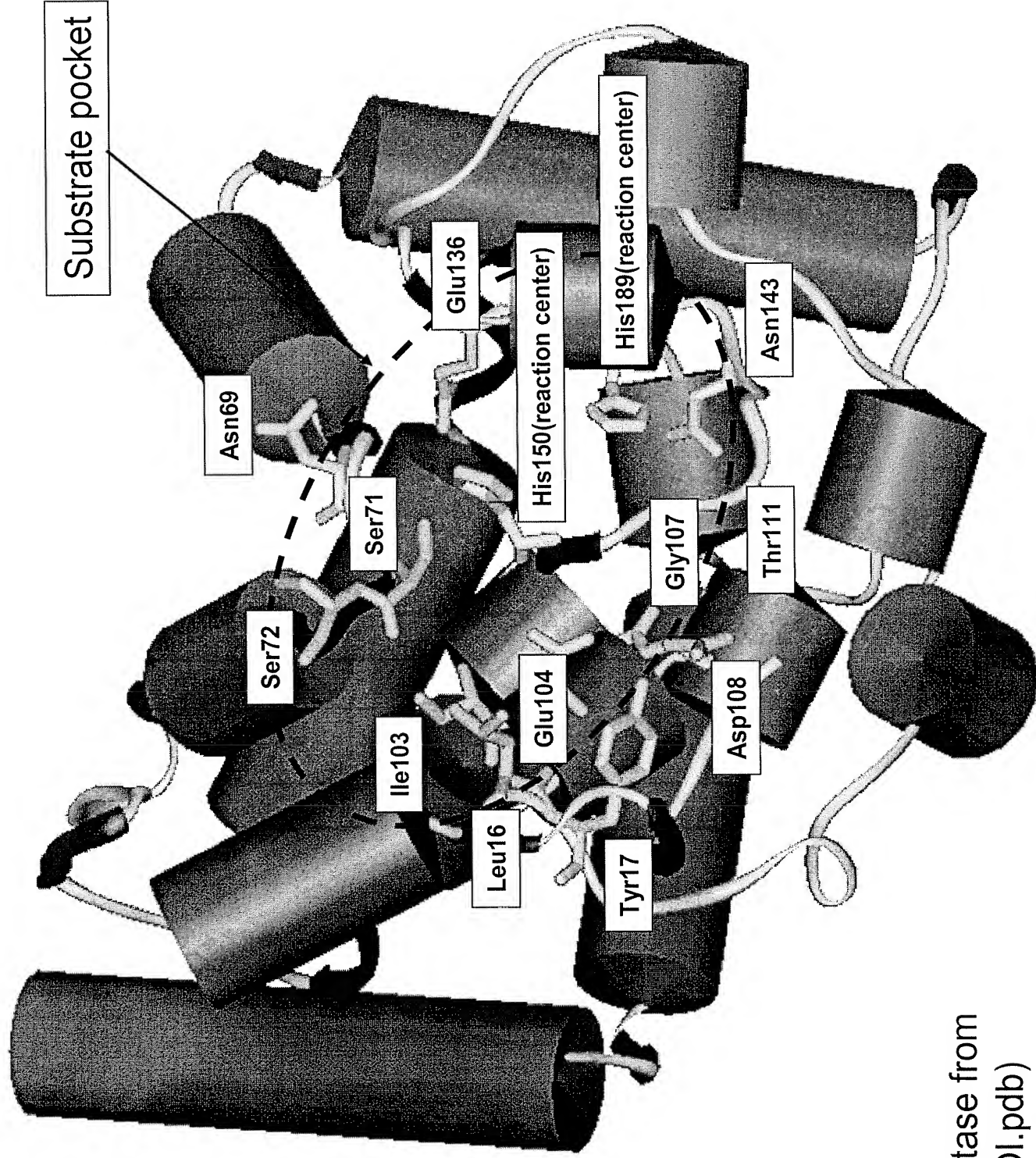


Fig.
B



2CST.pdb

Fig.
C



Acid phosphatase from
E. blattae(1EOL.pdb)

Fig. 1-2

Date : 2008.12.02

1st Amino Acid Sequence

File Name : Shigella flexneri.txt
Sequence Size : 249

2nd Amino Acid Sequence

File Name : NSAP Escherichia blattae_1E01.txt
Sequence Size : 231

Unit Size to Compare = 1
Pick up Location = 1

[84.4% / 231 aa] INT/OPT.Score : < 962/ 968 >

```
1' MKRQLFTLSIVGVFSLNTFASIPPGNDVTTKPDLYYLTNDNAIDSLALLPPPPQIGSIAF
    .* ... ***, *****, **, **, *****, *****, *****,
1" LALVATGNDTTTKPDLYYLNSEAINSLALLPPPPAVGSIAF

61' LNDQAMYEGRLLRNTERGKLAEDANLSSGGVANVFSAAFGSPITAKDSPELHKLLTNM
    *****, *****, *****, **, *****, **, *, *****,
43" LNDQAMYEQGRLLRNTERGKLAEDANLSSGGVANAFSGAFGSPITEKDAPALHKLLTNM

121' IEDAGDLATRSKEYMRIRPFAFYGVSTCNTKEQDTLSRNGSYPSGHTSIGWATALVLS
    *****, *****, *****, **, **, *****, *****,
103" IEDAGDLATRSKDHMRIRPFAFYGVSTCNTTEQDKLSKNGSYPSGHTSIGWATALVLA

181' EINPARQDTILKRGYELGDSRVICGYHWQSDVDAARIVGSAIVATLHSNPVFQAQLQKAK
    *****, **, *, *****, *****, *****, *****, *****, *****, **, **, *****,
163" EINPQRQNEILKRGYELGQSRVICGYHWQSDVDAARVVGSAVVATLHTNPAFQQQLQKAK

241' DEFANNQKK
    .***.***
223" AEFAQHQKK
```

Table.3-1

[Result]

Sample	time (hr)	products		Sample	time (hr)	products	
		area	conc.(mM)			area	conc.(mM)
2-deoxy-D-ribose	1	7218	0.7	2-deoxy-L-ribose	1	11227	1.1
	3	29247	2.9		3	29562	3.0
	6	64153	6.5		6	70285	7.1
	12	103710	10.4		12	96287	9.7
	24	95821	9.6		24	93370	9.4
D-ribose	1	24367	1.6	D-xylose	1	0	0
	3	70142	4.7		3	0	0
	6	112941	7.5		6	0	0
	12	202279	13.5		12	0	0
	24	147112	9.8		24	0	0
D-arabinose	1	14594	0.4	L-arabinose	1	7342	0.2
	3	35851	1.1		3	19823	0.6
	6	63462	1.9		6	38835	1.2
	12	112461	3.3		12	74587	2.2
	24	84555	2.5		24	53730	1.6
D-lyxose	1	0	0				
	3	0	0				
	6	0	0				
	12	0	0				
	24	0	0				

Fig.3-1

